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On the Transfer of Information from Old to New Chains of DNA Duplexes in Phage Lambda: Destruction of Heterozygotes*

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Abstract. The Watson-Crick model for DNA duplex duplication proposes that the two parental chains separate and that each directs the synthesis of a complementary chain with which it is found associated after the duplication act. Previous experiments have left unchallenged alternative models which propose that in any single act of duplication only one of the two parental chains provides information for the synthesis of both new chains. The models are operationally distinguishable since the former demands that heteroduplexes are destroyed by duplication while the latter anticipates their survival. We have shown for phage lambda that duplication destroys heterozygotes as predicted by the Watson-Crick model.

A stock of lambda containing a high frequency of heterozygotes at the cI locus was prepared by conducting a cross under conditions of depressed DNA synthesis. Particles in this lysate were permitted to duplicate a few times by adsorbing them to a lambda lysogen in a ^{15}N ^{13}C medium along with a heteroimmune lambda strain. Emerging lambda particles were separated according to density. The population of particles carrying DNA of parental density retained the initial high heterozygote frequency. Among particles which had duplicated, 80 per cent or more of the heterozygotes had disappeared.

Introduction. In the DNA-duplication scheme of Watson and Crick (1953) the two chains of a duplex separate from each other while each directs the synthesis of a chain complementary to itself. Other schemes for duplex duplication have been proposed. At least two of these (Kubitschek, 1964, and Jehle, 1965) have the feature that the information in both of the new chains is derived from only one of the old chains. These "master strand" models yield daughter duplexes comprising one old chain and one newly-synthesized chain. In this respect, they are indistinguishable from the Watson-Crick scheme. A clear distinction between Watson-Crick-type models and such "Master Strand" models could be made if one could trace information out of parental DNA chains into daughter molecules. Of course, such a test would be possible in principle only for the aberrant situation in which the two parental chains contain different information. In phage λ heterozygotes of the "heteroduplex" sort provide material appropriate for the test. The results of Heinemann and Meselson (1967) lend strong support to the assumption that heterozygotes arising as a result of

genetic recombination in phage λ are in fact heteroduplex in structure. Unpublished results of Weigle and D'Ari (personal communications), as well as unpublished results of our own, demonstrate that comparable yields of the two genotypes are recovered among the progeny from individual heterozygous particles. This is the expectation of the Watson-Crick scheme and rules out Master Strand models in which the *same strand* is master in each duplication act. However, the result does not distinguish between the Watson-Crick scheme and Master Strand models which permit *each strand* to serve as master during a cycle of intracellular phage growth. These alternatives are distinguished, however, by the predictions they make regarding the fate of the heterozygous condition. The Watson-Crick scheme demands that the heterozygosity disappear as a consequence of duplication; the Master Strand models predict that the products of duplication are one heterozygous daughter duplex and one homozygous duplex, unless, of course, the heterozygous region is made homozygous by some kind of DNA repair process. This paper demonstrates that duplication often and possibly always destroys heterozygosity in phage lambda. Experiments philosophically comparable to ours have been performed in the pneumococcus transformation system by Guerrini and Fox (1968). Their results also tend to favor Watson-Crick over Master Strand models.

Our experiment involved the following steps: (1) Production of a lambda lysate in a selectable portion of which the heterozygote frequency was high. (2) Infection of bacteria by this lysate under conditions which permit the recovery of mature phages after a small number of rounds of duplication; this infection was made in density-labeled bacteria in density-labeling medium. (3) Density-gradient separation of phages bearing DNA molecules which failed to duplicate from those bearing molecules whose duplication was attested to by their change in density. (4) Scoring of heterozygote frequencies among progeny phages bearing unduplicated and duplicated DNA molecules, respectively.

Materials and Methods. (1) **Preparation of a lambda lysate with a high heterozygote frequency ("Het lysate"):** *Sus*$^+$ recombinants from a cross of $cI_{26}susO_{29} \times susP_3$ (hereafter called *c susO* and *susP*, respectively) comprise about 2% of the progeny. Among these recombinants, selected by their ability to make plaques on amber-restrictive (*su*$^-$) bacteria, about 1% of the phages are heterozygous (c/c^+) as revealed by the mottled nature of the plaque. When the cross is performed under conditions of restricted DNA duplication, the heterozygote frequency among the recombinants is higher (Weigle, personal communication). Our Het lysate was produced as follows: Phage *c susO* and *susP* were adsorbed at 26° to the temperature-sensitive strain FA-22 (Fangman and Novick, 1968); total multiplicity was 10. This strain has impaired ability to duplicate its own DNA as well as that of lambda at temperatures above 26°. The infected cells were aerated in K medium at 26° for 140 min at which time they were transferred to 39°, and caffeine at a final concentration of 5 mg/ml was added. After 70 min at 39° the culture was chloroformed, and cellular debris was removed by low-speed centrifugation. The resulting lysate contained 2% *sus*$^+$ recombinants of which 50% were c^+, 25% were c/c^+ heterozygotes, and 25% were *c*. The three types were readily distinguished by plaque morphology on the *su*$^-$ indicator 594 (i^{434} *susO susP*).

(2) **Passage of Het lysate through a cycle of growth with a small number of duplications:** DNA duplication of homoimmune phage is repressed in lysogenic bacteria (Wolf and Meselson, 1963). Co-infection of the lysogen with a heteroimmune phage permits recovery of the chromosomes of the homoimmune phage among mature

progeny (Thomas and Bertani, 1964; and Ptashne, 1965). We used a su^+ lysogen carrying i^λ *sus*O *sus*P as immune host and the hybrid i^{434} *sus*O *sus*P as the heteroimmune "carrier" phage. This passage was carried out in a simple glucose medium; the glucose contained about 50% ^{13}C (Merck, Sharpe, and Dohme, Montreal); the nitrogen source was $^{15}NH_4Cl$ (Isomet).

(3) **Separation of phages according to isotope content:** Duplicated (hybrid and fully heavy), unduplicated, and unadsorbed phages were resolved by equilibrium centrifugation in a cesium formate—deuterium oxide gradient, a system chosen to provide greater resolution than does cesium chloride—water (Meselson, personal communication). Fractions were collected as successive drops emerging through a needle hole poked in the bottom of the centrifuge tube.

(4) **Scoring of Het frequencies:** Samples from the density gradient were plated on 594 (i^{434} *sus*O *sus*P). This indicator permits plaque formation only by i^λ sus^+ phages, and allows heterozygotes to be scored by inspection.

(5) **Media:**

M9: H_2O 1 liter, Na_2HPO_4 7 gm; KH_2PO_4 3 gm
NH_4Cl 1 gm, $MgSO_4$ 0.13 gm; glucose 1 gm
NaCl 0.8 gm; $FeCl_3$ 0.04 mgm

K: H_2O 500 ml, 3% Casamino acids (decolorized) 500 ml
Na_2HPO_4 7 gm, KH_2PO_4 3 gm, NH_4Cl 1 gm, Maltose 2 gm, $MgSO_4$ 0.65 gm, NaCl 0.8 gm

TM buffer: H_2O 1 liter, Trizma base 1.2 gm, $MgSO_4$, 0.13 gm adjust pH to 7.4.

BBL medium: BBL (Trypticase) 10 gm, NaCl 5 gm, H_2O 1 liter solidified with 10 gm agar for bottom layer and 6.5 gm agar for top layer.

Results. The ability of heterozygotes in the Het lysate to survive duplication was determined as follows: The *sus* permissive lysogen K12SH28 (i^λ *sus*O *sus*P) was grown in heavy M9 medium to a density of 1.5×10^8 cells/ml, spun down, and resuspended in TM buffer, at a concentration of 3×10^9 cells/ml. The culture was then infected with a light Het lysate at a total multiplicity of 0.1 and with light carrier phages at a multiplicity of 3. Adsorption was in 4×10^{-3} *M* KCN at room temperature for 30 minutes after which the cells were washed twice with TM buffer and resuspended in heavy medium. After 80 minutes at 37°, the cells were spun down, resuspended in D_2O and immediately lysed with chloroform. The lysate was then fractionated by centrifugation and the distribution of i^λ sus^+ phages determined (Fig. 1). The data for this experiment and for Experiment 1, performed on a different lysate, are summarized in Table 1 in a form which will facilitate discussion.

Discussion. The Watson-Crick model for DNA duplication predicts that among the yields from cells in which the infecting (light) DNA molecule was duplicated at least once, the frequency of heterozygotes will be zero. Master Strand hypotheses predict that the total number of heterozygotes does not change, although the frequency of heterozygotes diminishes in proportion to the factor by which the population size increases. If we assume that both light (L) chains and heavy (H) chains are subject to the same rules of maturation, we can calculate an *average* population size, x, by the relation $x = 2(R + 1)$ where R is the ratio of the number of phages in the H/H peak to the number in the H/L peak. For Experiment 1, R has the value 4.5–5.0 and for Experiment 2 the value 4.0–5.0 where the two numbers represent the extremes of values calculated corresponding to different definitions of peak width (3, 5, or 7 drops) and dif-

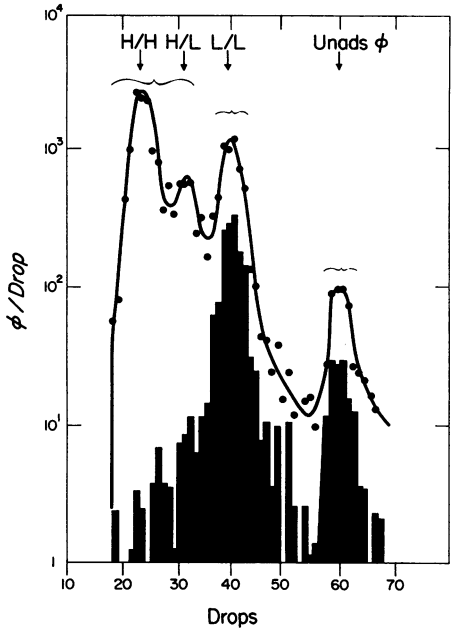

FIG. 1.—Distribution of heterozygotes among phages which have passed one cycle in an immune host. Three ml of lysate in D_2O was added to 2.6 gm cesium formate, and 3 ml of the solution was spun in a Spinco model L at 30,000 rpm for 30 hr at 24°. Single drops were collected in 1 ml broth each and measured (but variable) fractions of each collection tube were plated on 594(i^{434} *sus*O *sus*P) on a suitable number of plates. To avoid mottled plaques arising from accidental overlaps, we plated to give 100 or less plaques per plate. The shadowed histogram is the titer per 1 ml collection tube of mottled plaque formers. The data are those of Experiment 2 (see Table 1). BBL plates were used because they are good for plaque morphology (M. Meselson, personal communication).

ferent reasonable methods of resolution. The corresponding values of x are 11–12 for Experiment 1 and 10–12 for Experiment 2, The frequency of heterozygotes among duplicated phages is expected on Master Strand hypotheses, therefore, to be not lower than 22.7/12 = 1.9 per cent for Experiment 1 and 25.2/12 = 2.1 per cent for Experiment 2. The observed values (Table 1) are much less. Furthermore, we can explain the low but measurable frequency of heterozygotes in the duplicated peaks as the result of at least three factors: (*a*) overestimation due to the definition of duplicated peaks, (*b*) recombination between *L/L* heterozygote chromosomes and *H/H* i^{434} *sus*O *sus*P chromosomes, and (*c*) heterozygote formation by recombination in cells infected by Hets or by both *c sus*O and *sus*P phages.

TABLE 1. *Percentage of Hets.*

	Het lysate	Unadsorbed	Unduplicated peak	Duplicated peaks	Expected
Experiment 1	$22.7\left(\frac{325}{1431}\right)$	...	$23.5\left(\frac{439}{1862}\right)$	$0.3\left(\frac{51}{16,700}\right)$	1.9
Experiment 2	$25.2\left(\frac{197}{781}\right)$	$27.5\left(\frac{69}{251}\right)$	$23.6\left(\frac{536}{2276}\right)$	$0.45\left(\frac{36}{8,138}\right)$	2.1

The numbers of plaques counted are given in parentheses.

Per cent of heterozygotes among duplicated, unduplicated, and unadsorbed chromosomes following passage of a Het lysate through an immune host. Numbers in parentheses are the counts upon which the percentages are based. With respect to Experiment 2, "unadsorbed phages" is defined as the sum of phages in drops 58–63; the "unduplicated phages" are in drops 38–43; "duplicated phages" are in drops 19–33. Comparable definitions were made for Experiment 1. In order to minimize variations in mottled plaque frequency dependent upon plating conditions, for each experiment all the plates were plated on the same day on a single batch of plates and counted the following day. Each plate was counted by two people, one of whom was "blind" to the origin of the plate. (For the definition of "expected" percentage of heterozygotes, see *Discussion.*)

From the results reported in Table 1, we conclude that: (1) the frequency of heterozygotes is not altered by the centrifugation procedure (compare "unadsorbed phages" and Het lysate), (2) less than 10 per cent of the heterozygotes are destroyed if the DNA is not duplicated (compare Het lysate of "unadsorbed phages" and "unduplicated peak"), and (3) more than 80 per cent of the heterozygotes have disappeared after a few duplications.

Thus, there is a correlation between DNA duplication and heterozygote destruction. Unfortunately, this experiment cannot determine whether heterozygote destruction is the direct result of duplication as predicted by the Watson-Crick model or whether there is some kind of "heterozygote repair" that acts only during duplication or only on duplicated chromosomes and is efficient enough to destroy a substantial portion of heterozygotes.

Many friends gave freely of their advice and microbial strains during this, our first encounter with phage lambda. Of crucial importance were suggestions by Jean Weigle on the preparation of "Het lysate" and by Matt Meselson on density gradient centrifugation. George Streisinger provided sorely needed help and encouragement at a number of difficult moments.

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